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Feature Article

Research and Development of Pathogenic Microorganisms for the Control of Major Agricultural Pests

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Abstract

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Pathogenic microorganisms play a crucial role in agricultural pest management by suppressing pest populations through parasitism, infection, or toxin production. These microorganisms offer a sustainable and environmentally friendly alternative to chemical pesticides, contributing to integrated pest management (IPM) strategies. Insect-pathogenic microorganisms have been extensively studied and widely applied in biological control. These include bacterial pathogens such as *Bacillus thuringiensis*, entomopathogenic fungi (EPFs) like *Beauveria bassiana*, *Metarhizium anisopliae*, and *Cordyceps javanica*, as well as insect-pathogenic viruses such as nucleopolyhedrovirus (NPV). Due to their high specificity towards insect pests, environmental compatibility, and potential to reduce reliance on chemical pesticides, these microbial agents have become an integral part of IPM strategies. In recent years, advancements in genomics and biotechnology have significantly accelerated the selection of highly effective pathogenic strains and the elucidation of their virulence mechanisms. Whole-genome sequencing and transcriptomic analyses have facilitated the precise identification of key genes involved in pathogenicity, enabling further exploration of toxin production, parasitic capabilities, and host adaptability. Additionally, studies on specific enzymes, such as chitinases and proteases, as well as secondary metabolites like fungal toxins and bacterial exoproteins, have contributed to enhancing the infection efficiency and insecticidal activity of these microorganisms, expanding their application potential. Despite the numerous advantages of microbial control, including environmental safety, high specificity, and minimal residue concerns, several challenges remain in practical applications. These include the environmental adaptability of microbial agents, the evolution of host resistance, and issues related to formulation stability and field efficacy. Overall, microbial control technologies play a vital role in modern agriculture. With continuous advancements in biotechnology and the development of novel application strategies, these technologies are expected to become a cornerstone of pest management, promoting sustainable agriculture and global food security.

Key words: Entomopathogens, Microbial pesticides, Bacteria, Fungi, Viruses.

INTRODUCTION

Pest infestations are an inevitable challenge in agricultural cultivation. Synthetic chemical pesticides have long been the primary tool for pest management due to their rapid action, high efficacy, and ease of application. They also remain an essential component of integrated pest management (IPM). However, concerns over their environmental toxicity, negative impacts on non-

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target organisms, and the increasing prevalence of pesticide resistance (Ku *et al*. 1994; Tabashnik 1994; Gao *et al*. 2012; Adesanya *et al*. 2018; Lin 2019; Jensen 2000; Bird *et al*. 2022; Bass & Nauen 2023) have led to a growing emphasis on the use of microbial pathogens for pest control.

In natural ecosystems, insects encounter a diverse range of microorganisms, including environmental microbes and endogenous symbionts. Some of these microbes can infect and cause disease in insects, ultimately leading to their death. Others, particularly endophytic microorganisms, play dual roles by suppressing plant pathogens and promoting plant growth. The most commonly used microbial pathogens for pest management fall into three main categories: bacteria, fungi, and viruses.

This review provides an overview of the applications of key microbial pathogens in agricultural pest management, highlighting their advantages as well as the challenges associated with their implementation. The scientific names of bacteria, fungi, and insects mentioned in this review follow the current valid nomenclature according to the International Code of Nomenclature of Prokaryotes (Parker *et al*. 2019), the International Code of Nomenclature for algae, fungi, and plants (Turland *et al*. 2018), and the International Code of Zoological Nomenclature (ICZN 1999), respectively. Taxonomic information was verified using the List of Prokaryotic names with Standing in Nomenclature (LPSN, https://lpsn.dsmz.de), Fungal Names (https://nmdc.cn/fungalnames), Catalogue of Life (https://www.catalogueoflife.org), and the Global Biodiversity Information Facility (GBIF, https://www.gbif.org).

BACTERIAL MICROBIAL AGENTS IN AGRICULTURAL PEST BIOCONTROL

Bacteria-based microbial formulations are widely used in agricultural pest control. Their production and field performance- affected by drying, heating, storage, humidity, temperature, and ultraviolet (UV) exposure- largely depend on the bacteria's ability to form spores. Pathogenic bacteria are therefore classified into three groups: (1) endospore-formers, such as *Bacillus*, *Clostridium*, and *Paenibacillus*; (2) exospore-formers, e.g., *Streptomyces*; and (3) non-spore-formers, including *Pseudomonas*, *Photorhabdus*, *Chromobacterium*, *Xenorhabdus*, *Serratia*, and *Yersinia* (Beskrovnaya *et al*. 2021; Sabbahi *et al*. 2022).

Bacillus thuringiensis

Bacillus thuringiensis Berliner, 1915 (Bt) belongs taxonomically to the phylum *Bacillota*, class *Bacilli*, order *Caryophanales*, family Bacillaceae, and genus *Bacillus*. Bt is a Gram-positive soil bacterium capable of producing insecticidal δ-endotoxins, which exhibit high lethality against various agricultural pests, including insects from the orders Lepidoptera, Coleoptera, and Diptera.

Under favorable environmental conditions, Bt spores germinate, and vegetative cells proliferate. However, under unfavorable conditions, during the stationary phase, Bt forms parasporal crystalline inclusions composed of Cry and Cyt proteins (Suzuki *et al*. 2004). Additionally, during the vegetative growth phase, Bt synthesizes and secretes insecticidal toxins into the growth medium, such as vegetative insecticidal proteins (Vip) and secreted insecticidal proteins (Sip) (Palma *et al*. 2014).

Cry proteins (δ -Endotoxins)

Cry proteins, also known as δ-endotoxins, are pore-forming toxins (PFTs) that are water-soluble proteins. These proteins undergo structural changes that allow them to insert into the host membrane, forming lytic pores that disrupt the integrity of the host cell membrane (Gonzalez *et al*. 2008; Sanchis & Bourguet 2008). Cry proteins are ingested by insects in the form of parasporal crystalline inclusions. In the alkaline environment of the insect midgut, these crystals are solubilized and hydrolyzed into protoxins, which subsequently bind to specific receptors on the midgut epithelial cells.

This binding triggers pore formation, disrupts osmotic balance, and ultimately leads to cell death (Fig. 1). The widespread destruction of midgut epithelial cells compromises the integrity of the gut lining, allowing Bt and other gut bacteria to invade the nutrient-rich hemocoel, proliferate, and cause septicemia, resulting in insect mortality (Raymond *et al*. 2010). Due to its efficacy, Bt-based products containing spores and crystalline toxin mixtures have been commercialized for controlling various insect larvae, including caterpillars, beetles, and mosquitoes. Additionally, transgenic crops expressing Bt toxin genes, such as cotton and maize, have been cultivated in many countries. These genetically modified (GM) crops have been widely adopted globally, effectively reducing insect damage and minimizing the need for chemical pesticides (Raymond *et al*. 2010).

Evolution of cry protein classification

Cry proteins exhibit high specificity toward different insect species, primarily due to their selective binding to surface proteins on the microvilli of midgut epithelial cells in insect larvae. This binding specificity determines their insecticidal activity. Initially, Cry proteins were classified based on their target insect groups. Among them, Cry1, Cry2, and Cry9 exhibit toxicity against Lepidoptera. Cry2 also targets both Lepidoptera and Diptera. Cry3, Cry7, Cry8, and Cry1Ia are effective against Coleoptera. Cry4, Cry10, Cry11, and Cyt exhibit toxicity toward Diptera. Cry5, App6, and Cry12 are toxic to nematodes and the two-spotted spider mite (Krieg *et al*. 1983; Höfte & Whiteley 1989; Haffani *et al*. 2001; Wei *et al*. 2003; Ruiz de Escudero *et al*. 2007; Hernández-Martínez *et al*. 2008; Hernández-Soto *et al*. 2009; Domínguez-Arrizabalaga *et al*. 2020; Valtierra-de-Luis *et al*. 2020; Guo *et al*. 2022; Unzue *et al*. 2022).

As research on Cry proteins advanced and became increasingly complex, a revised classification system was introduced in 1998, categorizing proteins solely based on amino acid sequence similarity (Crickmore *et al*. 1998). This

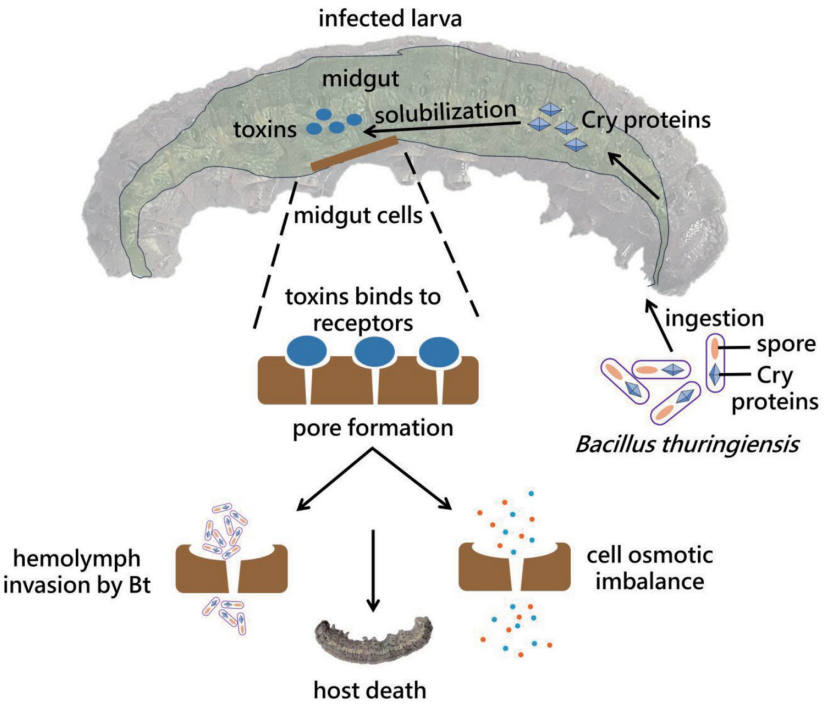

Fig. 1. Mode of action of *Bacillus thuringiensis* in Lepidoptera.

four-tiered nomenclature system groups proteins with more than 45% sequence similarity into the same primary classification (e.g., Cry1, Cry2). Proteins with less than 78% similarity within a primary group are further assigned to different secondary levels (e.g., Cry1A, Cry1B). The tertiary level is designated for proteins within a secondary group that share less than 95% sequence similarity (e.g., Cry1Aa, Cry1Ab). Finally, the quaternary level differentiates proteins within a tertiary classification that exhibit over 95% sequence identity (e.g., Cry1Aa1, Cry1Aa2). Beyond Cry proteins, which are insecticidal toxins derived from crystalline inclusions, the classification also includes Cyt proteins, which exhibit *in vitro* cytolytic activity and are specifically toxic to dipteran insects, as well as Vip proteins, which are secreted by Bt during the vegetative growth phase (Estruch *et al*. 1996).

As further research expanded the discovery of toxic proteins from Bt and other bacterial species, the nomenclature system was updated to classify proteins based on full-length sequence alignment, with adjusted similarity thresholds of 45%, 76%, and 95%. This revision led to the definition of sixteen structural classes of pesticidal proteins, including crystalline and insecticidal (Cry) proteins, cytolytic proteins (Cyt), Vip, proteins related to the ADP-ribosyltransferase active component of binary toxins (Vpa), proteins related to the binding component of binary toxins (Vpb), beta pore-forming pesticidal proteins (Tpp and Mpp), aegerolysin like pesticidal proteins (Gpp), alpha helical pesticidal proteins (App), sphaericolysin like pesticidal proteins (Spp), proteins related to the "Makes Caterpillars Floppy" toxins (Mcf), proteins related to the Mtx1 toxin (Mtx), proteins related to the *Photorhabdus* insect-related toxin (Pra and Pra), pesticidal proteins that are part of the Membrane Attack Complex/Perforin superfamily (Mpf), and a holding class for pesticidal proteins with currently uncharacterized structures (Xpp) (Crickmore *et al*. 2021). This refined classification system continues to facilitate the systematic identification and characterization of novel pesticidal proteins, contributing to advancements in biocontrol strategies.

Subspecies of *B. thuringiensis* and their insecticidal specificity

Bt is classified into approximately 30 subspecies based on flagellar antigenic properties and various biochemical tests. These subspecies exhibit different insecticidal activities against specific insect orders. For example, Bt subsp. *aizawai* (Bta) and Bt subsp. *kurstaki* (Btk) are highly toxic to lepidopteran pests such as *Plutella xylostella* (Linnaeus, 1767) and *Agrotis ipsilon* (Hufnagel, 1766) (Iqbal *et al*. 1996; Mashtoly *et al*. 2011). Bt subsp. *san diego* and Bt subsp. *tenebrionis* are effective against coleopteran insects, while Bt subsp. *israelensis* (Bti) has high toxicity against dipteran pests, including mosquitoes (McPherson *et al*. 1988; Bauer 1990; Ben-Dov 2014).

Advantages and challenges of *B. thuringiensis* applications

Bt-based biopesticides offer several advantages, including safety for humans and non-target organisms, reduced pesticide residues in food, conservation of natural enemies, and promotion of biodiversity in managed ecosystems. However, their application faces challenges such as the potential development of insect resistance and environmental factors affecting Bt activity. To address these limitations, researchers are actively developing novel Bt strains with enhanced insecticidal activity and integrating Bt with other biocontrol strategies, including entomopathogenic fungi (EPFs), viruses, and nematodes, to improve pest management efficacy.

The first commercial Bt cotton was introduced in the United States in 1996, effectively controlling three major lepidopteran pests: *Heliothis virescens* Fabricius, 1777, *Helicoverpa zea* (Boddie, 1850), and *Pectinophora gossypiella* (Saunders, 1844) (Mendelsohn *et al*. 2003). Subsequently, GM crops expressing Bt proteins, such as maize, soybean, and eggplant, have

been widely adopted, with their cultivation areas expanding annually (Meissle *et al*. 2022).

Effects of Bt crops on non-target organisms

The potential impact of Bt crops on non-target insects has been a major concern. A laboratory-scale study first reported that monarch butterfly (*Danaus plexippus* (Linnaeus, 1758)) larvae experienced mortality after exposure to Bt maize pollen, raising concerns about the potential risks of Bt toxins to non-target species (Losey *et al*. 1999). However, further research has demonstrated that the insecticidal proteins used in commercial GM crops (Cry and Vip) do not pose harmful effects on pollinators such as honey bees (*Apis mellifera* Linnaeus, 1758) (Malone & Burgess 2009). A study comparing two Bt maize varieties—one expressing a single Bt protein and another expressing three Bt proteins- has found no adverse effects on honey bee larval survival or prepupal weight (Hendriksma *et al*. 2011). A meta-analysis summarizing data from 12 bibliographic databases, 17 specialized websites, 78 review articles, and over 7,200 records from 233 experiments and 120 studies concluded that Bt maize had minimal impact on non-target invertebrate communities in maize fields (Meissle *et al*. 2022).

Other bacterial pathogens for agricultural pest control

Among bacterial pathogens used in agricultural pest management, *Paenibacillus popilliae* (Dutky 1940) Pettersson *et al*. 1999 (formerly *Bacillus popilliae*) was the first microbial agent registered as an insecticide. It is primarily used to control the Japanese beetle (*Popillia japonica* Newman, 1838) in turfgrass (Adams & Wheeler 1946; Arthurs & Dara 2019). Additionally, *Chromobacterium subtsugae* Martin *et al*. 2007 and *Burkholderia* spp. have a broad host range and have been developed as broad-spectrum insecticides and miticides, effective against whiteflies, aphids, scale insects, and spider mites (Shannag & Capinera 2018; Golec *et al*. 2020; Shannag 2025).

Bacteria used in agricultural pest control are often isolated from environmental sources such as soil and plants. However, this traditional screening approach is time-consuming, labor-intensive, and has low efficiency. Examples of bacterial biopesticides obtained through this method include *B. thuringiensis*, *C. subtsugae*, *Burkholderia* spp., and *Pseudomonas putida* (Trevisan, 1889) Migula, 1895 (Starnes *et al*. 1993; Martin *et al*. 2007; Aksoy *et al*. 2008). In recent years, some studies have focused on isolating bacterial pathogens directly from infected insects or mites. For example, *Bacillus velezensis* Ruiz-García *et al*. 2005 w1 was isolated from diseased *Tetranychus urticae* Koch, 1836 (Li *et al*. 2019). Another notable example is *Photorhabdus luminescens* (Thomas and Poinar, 1979) Boemare *et al*. 1993, which was isolated from the gut of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976. This bacterium exists in a symbiotic relationship with the nematode, aiding in host infection and insect mortality (Boemare *et al*. 1993; Hsieh *et al*. 2023).

Synergistic effects of pathogenic micro-organisms

The combination of pathogenic micro-organisms with complementary modes of action can enhance insecticidal efficacy and delay the development of pest resistance. Recent studies have demonstrated notable synergistic effects among bacteria, fungi, and viruses used in pest management. For instance, *P. luminescens* (Pl) combined with Btk exhibited strong synergism against *Spodoptera frugiperda* (Smith, 1797), reducing the Bt dosage required for comparable mortality by up to fivefold. Higher proportions of Bt led to increased *P. luminescens* loads in the hemocoel and more severe midgut damage, indicating that Bt promotes *P. luminescens* invasion and augments its virulence (Chang *et al*. 2024).

Similarly, synergistic interactions between NPVs and Bt have been reported against *A. ipsilon*. *Mamestra brassicae* (Linnaeus, 1758)

multiple nucleopolyhedrovirus (MbMNPV) showed the highest virulence, while combinations of *Spodoptera litura* (Fabricius, 1775) MNPV (SlMNPV) and Bt produced synergistic effects, resulting in elevated larval mortality and altered survival dynamics compared to single-pathogen treatments. In contrast, other NPV–Bt combinations exhibited additive or variable interactions depending on strain and concentration (Mao *et al*. 2025).

Collectively, these findings highlight that inter-microbial synergy- whether between bacteria and viruses or among bacterial species- can substantially enhance biocontrol performance. Understanding these interactions provides a promising foundation for developing optimized microbial formulations that improve field efficacy while supporting sustainable pest management.

Future research directions in bacteria-based pest control

The success of microbial pest control strategies depends on their adaptability to environmental conditions, formulation stability, and synergy with other pest management measures. Future research should focus on: (1) Enhancing the virulence and speed of action of pathogenic strains to improve their effectiveness against target pests. (2) Improving the adaptability of microbial pathogens to extreme environmental conditions, such as low temperatures and arid climates, to expand their application range. (3) Increasing production efficiency for large-scale applications, ensuring the economic feasibility and sustainable supply of microbial pesticides. (4) Developing improved formulations to enhance ease of use, environmental persistence, and shelf life. (5) Exploring how insect-pathogenic bacteria can be effectively integrated into IPM systems, including their interactions with environmental factors and other IPM components. (6) Conducting more precise assessments of the environmental benefits of insect-pathogenic bacteria to promote their sustainable development and application. (7) Encouraging broader acceptance and adoption of microbial pesticides among farmers and the general public, increasing their market penetration.

In summary, bacteria-based microbial pesticides play a crucial role in agricultural pest control. However, their application requires careful consideration of multiple factors to achieve optimal pest management outcomes. Furthermore, certain insect-pathogenic bacteria, such as Bt, not only serve as agricultural insecticides but also produce a variety of bioactive compounds with diverse applications (Salehi Jouzani *et al*. 2025). These include plant disease suppression, plant growth promotion, biofuel production, bioplastic synthesis, nanoparticle fabrication, food preservation, cancer treatment, and heavy metal bioremediation (Salehi Jouzani *et al*. 2025). These multifunctional properties underscore the value of bacterial biopesticides not only in pest control but also as essential resources in sustainable agriculture and biotechnology.

FUNGI IN AGRICULTURAL PEST BIOCONTROL

As of 2023, a total of 27 species of EPFs have been registered as biopesticides worldwide (Jiang & Wang 2023). Among these, the most commonly applied EPFs in agricultural pest control belong to the genera *Beauveria*, *Metarhizium*, *Cordyceps*, *Hirsutella*, *Verticillium*, *Lecanicillium*, and *Paecilomyces*. These fungi not only serve as effective agents for pest control but, in some cases, can also act as endophytic insect pathogenic fungi (EIPFs). EIPFs colonize plant tissues, where they promote plant growth and enhance the plant's immune system, contributing to improved plant health (Kabaluk & Ericsson 2007; Gupta *et al*. 2022).

Among these genera, *Beauveria* spp. is the most widely used for pest management due to its ability to produce more than ten insecticidal metabolites, making it a valuable tool for controlling a broad range of pests (Zhang *et al*. 2020; Xing *et al*. 2024). *Metarhizium* spp., commonly found in soil, plant roots, and insects, exhibit a broad host range and are a key model

for studying the co-evolution of fungal virulence in insects and plants (Sheng *et al*. 2022). During the later stages of infection, insects infected with *Metarhizium* sp. show the presence of destruxin, an insecticidal metabolite produced by the fungus (Suzuki *et al*. 1970; Liu & Tzeng 2012). *Cordyceps* fungi, which are parasitic on insects or arthropods, are effective in controlling pests such as whiteflies, aphids, banana root borers (*Cosmopolites sordidus* (Germar, 1823)), and spider mites (Negrete González *et al*. 2018; Nishi *et al*. 2023; Wang *et al*. 2023). During their invasion of insect hosts, *Cordyceps* fungi secrete various enzymes and small insecticidal toxins that contribute to their pest control capabilities (Wang *et al*. 2023; Xing *et al*. 2024). Below is a detailed description of the more commonly applied species in pest control.

Beauveria bassiana

Beauveria bassiana (Bals.-Criv.) Vuill., 1912, an insect pathogenic fungus, is classified within the phylum Ascomycota, class Sordariomycetes, order Hypocreales, family Cordycipitaceae, and the genus *Beauveria*. *B. bassiana* is a widely applied biopesticide in agricultural pest control, capable of infecting over 1,000 insect species and exhibiting toxic effects against a broad range of agricultural pests (Araújo & Hughes 2016; Vivekanandhan *et al*. 2024). Notably, it is relatively safe for non-target organisms and vertebrates (Zimmermann 2007). In addition to its use as a biocontrol agent, *B. bassiana* can also function as an endophytic fungus, colonizing plant tissues without causing symptoms, and even promoting host plant growth (Lopez & Sword 2015; McKinnon *et al*. 2017; Afandhi *et al*. 2019; Grabka *et al*. 2022; Liu *et al*. 2022).

Infection process and mechanisms of *B. bassiana*

Upon infecting insects, *B. bassiana* attaches its conidia (asexual spores) to the insect cuticle, where the conidia germinate and form an appressorium. This structure combines biochemical and mechanical pressures to penetrate the cuticle and enter the insect's hemocoel, where the fungus produces mycelium that invades the insect's tissues. The fungus then generates blastospores, eventually leading to the host's loss of mobility and death. During this process, mycelium grows from the insect's intersegmental membranes, eventually covering the insect's body and producing a mass of white, powdery spores. These spores can spread to other healthy insects, continuing the cycle of infection (Fig. 2). *B. bassiana* infections rely on multiple mechanisms, including the production of a variety of toxic secondary metabolites. These metabolites enhance fungal invasion and suppress the insect's immune defense system (Pedrini 2022). More than ten insecticidal metabolites have been identified, including nonribosomal peptides and polyketides such as beauvericin, bassianolide (cyclooligomeric nonribosomal peptides), beauverolides (cyclic peptides), oosporein (dibenzoquinone), bassiatin (diketomorpholine), and tenellin (2-pyridone) (Zhang *et al*. 2020). While the exact roles of these secondary metabolites remain unclear, they are generally correlated with the virulence of fungal strains (Butt *et al*. 2016; Zhang *et al*. 2020; Pedrini 2022). For example, Vivekanandhan *et al*. (2024) isolated secondary metabolites produced by *B. bassiana* and evaluated their toxicity against *Tuta absoluta* (Meyrick, 1917) larvae, and found that 9,10-octadecadienoic acid exhibited the strongest toxicity against the larvae. In a study by Tsai *et al*. (2006), the pathogenicity of eight local Taiwanese *B. bassiana* strains was compared, revealing significant differences in their effectiveness against pests such as the diamondback moth (*P. xylostella*) and beet armyworm (*Spodoptera exigua* (Hübner, 1808)).

Endophytic characteristics and plant growth promotion

The endophytic nature of *B. bassiana* enables it to colonize plant tissues, promote plant growth, and improve plant health without causing harm. For example, it has been shown to enhance the antioxidant activity of lettuce

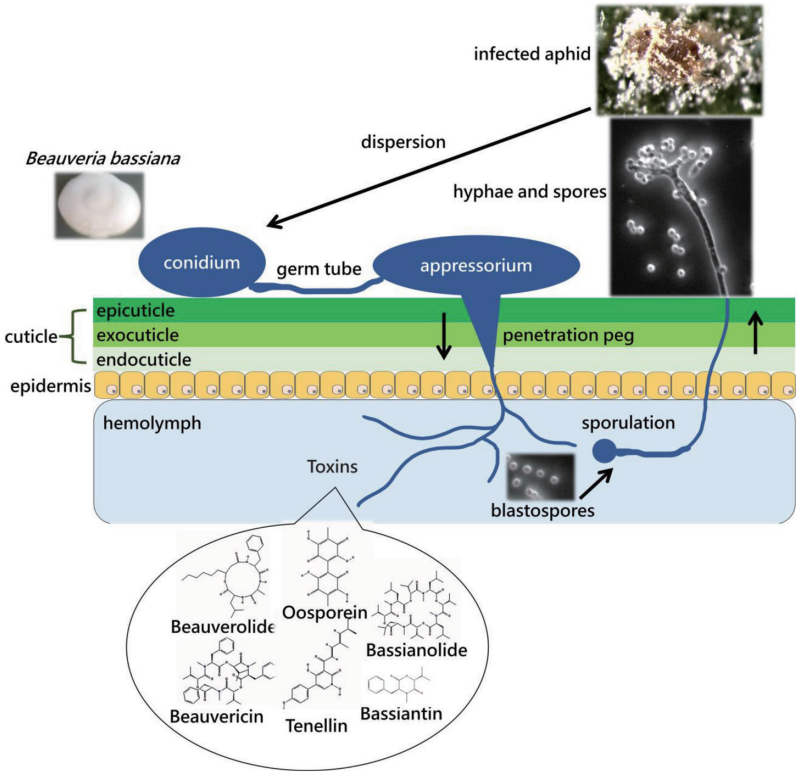

Fig. 2. Mode of action of *Beauveria bassiana* in aphids.

(*Lactuca sativa* L.) and can even be transmitted vertically through seeds in crops like cotton (*Gossypium hirsutum* L.) and common bean (*Phaseolus vulgaris* L.) (Quesada-Moraga *et al*. 2014; Lopez & Sword 2015; Afandhi *et al*. 2019; Macuphe *et al*. 2021). These characteristics open new avenues for IPM strategies, making *B. bassiana* a key player in sustainable agriculture. Continued research into its endophytic traits will be essential in developing innovative pest management strategies that align with ecological principles.

Challenges and future directions

B. bassiana, as a biocontrol agent, can be integrated with other pest management strategies in the integrated management of agricultural pests. For instance, in the control of the coffee berry borer (*Hypothenemus hampei* (Ferrari, 1867)), the combination of *B. bassiana* and CMU-C1 lure-modified traps (Krutmuang *et al*. 2023) has been shown to reduce pest populations in coffee plantations. Different strains of *B. bassiana* exhibit variations in growth characteristics, virulence, and environmental adaptability, necessitating the selection of suitable strains based on the target pest and environmental conditions. However, large-scale application of *B. bassiana* still faces challenges related to inconsistent efficacy. Future research should focus on enhancing its endophytic potential, improving formulation development, and optimizing application techniques to strengthen its role in IPM.

Metarhizium anisopliae

The genus *Metarhizium*, commonly known as EPFs, belongs to the phylum Ascomycota, class Sordariomycetes, order Hypocreales, and family Clavicipitaceae. Initially, the genus contained only four species; however, advancements in DNA sequencing techniques

have expanded its classification to include over 30 species (Bischoff *et al*. 2009; Brunner-Mendoza *et al*. 2019). The host range of different *Metarhizium* species varies significantly. For example, *Metarhizium anisopliae* (Metschn.) Sorokīn, 1883 has a broad host range, infecting more than 200 insect species, while *M. acridum* is highly specialized, primarily targeting Orthoptera (Gao *et al*. 2011; Brunner-Mendoza *et al*. 2019). Some species within this genus exist as saprophytic fungi in the soil, serving as rhizosphere microbes, while others function as endophytic fungi colonizing plant tissues or as potent insect pathogens (Hu & Leger 2002; Wyrebek *et al*. 2011; Steinwender *et al*. 2015; Moonjely & Bidochka 2019).

M. anisopliae: A model species

M. anisopliae is the type species of the *Metarhizium* genus and is a well-known entomopathogenic fungus with a broad host range, infecting over 200 insect species, including many agricultural pests (Lomer *et al*. 1997; Gao *et al*. 2011; Contreras *et al*. 2014; Gabarty *et al*. 2014; Perumal *et al*. 2024). It also exhibits endophytic behavior, colonizing plant tissues and promoting plant growth (González-Pérez *et al*. 2022; Liu *et al*. 2022). The species was initially isolated from the beetle *Anisopliae austriaca* (Herbst, 1783) and was originally named *Entomophthora anisopliae*. However, subsequent studies based on conidial morphology and DNA sequence data led to its reclassification as *M. anisopliae* (Brunner-Mendoza *et al*. 2019).

Infection process of *M. anisopliae*

The infection process of *M. anisopliae* closely resembles that of *B. bassiana* and consists of six key stages: (1) Attachment of Conidia: The fungal conidia adhere to the insect cuticle. (2) Germination and Growth: The conidia germinate and develop. (3) Appressorium Formation: A specialized infection structure called the appressorium is formed. (4) Cuticle Penetration: The fungus penetrates the insect cuticle and invades the hemocoel. (5) Fungal Proliferation: Mycelium spreads throughout the insect's tissues, producing blastospores, leading to host immobilization and eventual death. (6) Sporulation and Transmission: Mycelium emerges from the insect's intersegmental membranes, covering the body with powdery white spores, which are then disseminated to infect new hosts (Aw & Hue 2017; Peng *et al*. 2022).

Molecular mechanisms underlying infection

Early-stage attachment and conidial germination

During the initial infection stage, *M. anisopliae* conidia attach to the insect cuticle. The conidial surface contains hydrophobic proteins that facilitate adhesion to the host's hydrophobic cuticle (Tseng *et al*. 2014). Once attached, cuticular lipids trigger conidial germination, with carbon and nitrogen sources serving as essential elements for spore activation (Ment *et al*. 2010).

Appressorium formation and cuticle penetration

The development of the appressorium is crucial for host infection. The *ODC1* gene encodes ornithine decarboxylase, which is involved in conidial germination and appressorium formation, while the *Mpl1* gene encodes MPL1, which regulates lipid homeostasis and appressorium differentiation (Wang & Leger 2007b; Pulido *et al*. 2011). Protein kinase A (PKA) plays a role in appressorium and penetration peg differentiation by regulating glycerol permeability under hypoosmotic conditions, thereby maintaining turgor pressure within the appressorium (Fang *et al*. 2009). The *Mos1* gene encodes an osmosensor, which aids in osmoregulation (Wang *et al*. 2008). The high turgor pressure generated enables the fungus to apply mechanical force, facilitating cuticle penetration (Lovett & Leger 2015). In addition, the conidial appressorium secretes the MAD1 protein, which binds to the host insect cuticle (Wang & Leger 2007a).

Enzymatic degradation of the cuticle

The penetration process of EPFs involves the action of multiple hydrolytic enzymes that degrade the insect cuticle. These enzymes include elastase, subtilisins (Pr1 and Pr2), trypsins, chymotrypsins, carboxypeptidases, chitinases, and lipolytic enzymes (Joshi & Leger 1999; Bagga *et al*. 2004; Beys da Silva *et al*. 2010; Santi *et al*. 2010a, 2010b; Schrank & Vainstein 2010; Aw & Hue 2017). The expression of these enzymes varies depending on the host species. For instance, different isoforms of elastase (Pr1A–K) may be involved in the early infection stages of *M. anisopliae* and play a role in host recognition. Notably, the Pr1I protease is expressed during the infection of the cotton stainer bug (*Dysdercus peruvianus* Guérin-Méneville, 1831) (Santi *et al*. 2010b). In contrast, Pr1J is significantly upregulated in infections of the cockroach (*Blaberus giganteus* (Linnaeus, 1758)) and the gypsy moth (also called spongy moth) (*Lymantria dispar* (Linnaeus, 1758)) but not in the Japanese beetle (*P. japonica*) (Freimoser *et al*. 2005). These variations in enzyme expression are associated with *M. anisopliae*'s ability to recognize specific hosts.

In culture media simulating infections of *D. peruvianus* and cattle tick (*Rhipicephalus microplus* (Canestrini, 1888)) by *M. anisopliae*, chitinases were detected. Although these chitinases shared the same molecular weight, they exhibited differences in isoelectric points (pI), suggesting that *M. anisopliae* may secrete distinct chitinase isoforms depending on the host species (Santi *et al*. 2010b). Furthermore, mutant strains of *M. anisopliae* lacking chitinase genes exhibited significantly lower virulence against *D. peruvianus*, highlighting the crucial role of chitinase in fungal pathogenesis (Boldo *et al*. 2009).

Destruxins: Key secondary metabolites of *M. anisopliae*

The most common secondary metabolites found in the fermentation broth of *M. anisopliae* are a family of cyclic peptide toxins known as destruxins. These compounds are cyclic hexadepsipeptides composed of an α-hydroxy acid and five amino acid residues (Hsiao & Ko 2001; Pedras *et al*. 2002; Wang *et al*. 2012). Destruxins are classified into five main groups (A to E) based on their chemical structures (Pedras *et al*. 2002). As of 2010, a total of 38 different destruxins had been identified, with most of them being secondary metabolites of *M. anisopliae* (Schrank & Vainstein 2010). Among these, destruxins A, B, and E exhibit significant insecticidal activity (Sree *et al*. 2008). Destruxin B was the first to be synthesized through organic synthesis (Kuyama & Tamura 1965). The biosynthesis of destruxins was first identified in *M. anisopliae* and is regulated by a non-ribosomal peptide synthetase (NRPS) gene cluster (Wang *et al*. 2012). Destruxins exert their toxic effects by opening cellular Ca^{2+} channels, disrupting the Ca^{2+}/calmodulin signaling pathway. This process leads to an influx of extracellular Ca^{2+}, causing insect muscle tetanic contraction, paralysis, and ultimately death. Additionally, destruxins suppress the insect immune response, thereby enhancing fungal virulence (Ruiz-Sanchez *et al*. 2010; Wang *et al*. 2012).

Conclusion

Metarhizium fungi hold significant application value in the fields of entomopathology and biological control. The genus is diverse, ranging from the broad host-range *M. anisopliae* to the host-specific *M. acridum*, demonstrating different ecological adaptation strategies. Additionally, these fungi are not only efficient insect pathogens but also possess potential as endophytes and plant growth promoters, making them multifunctional biocontrol agents. Their infection mechanism involves attachment, spore germination, formation of infection structures, enzymatic degradation, and the toxic effects of secondary metabolites such as destruxins, illustrating a complex and precise pathogenic process. However, despite extensive research revealing their biological characteristics, *Metarhizium* still faces numerous challenges in practical applications. With advancements

in genomics, synthetic biology, and ecology, future research will help optimize the application potential of *Metarhizium*, promoting its practical use in sustainable agriculture and IPM.

Cordyceps javanica

The entomopathogenic fungus *Cordyceps javanica* (Bally) Kepler, Shrestha & Spatafora, 2017 belongs to the phylum Ascomycota, class Sordariomycetes, order Hypocreales, family Cordycipitaceae, and genus *Cordyceps*. The genus *Cordyceps* comprises 629 known species, all of which parasitize insects or other arthropods (Qu *et al*. 2022). Among them, several species are commonly used in biopesticides, including *C. javanica*, *Cordyceps fumosorosea* (Wize) Kepler, Shrestha & Spatafora, 2017, *Cordyceps farinosa* (Holmsk.) Kepler, Shrestha & Spatafora, 2017, and *Cordyceps cateniannulata* (Z.Q. Liang) Kepler, Shrestha & Spatafora, 2017 (Jiang & Wang 2023). Notably, *C. javanica* has the most extensive genomic data available among these species (https://www.mycobank.org).

Infection process of *C. javanica* in *Acyrthosiphon pisum*

The infection process of *C. javanica* in the pea aphid (*Acyrthosiphon pisum* (Harris, 1776)) follows a well-defined sequence of events. After *C. javanica* conidia adhere to the insect cuticle, they germinate and form germ tubes and penetration pegs. Mucilage matrices can be observed at the interface between the germ tubes and the cuticle. The germ tubes develop into hyphae, with some penetrating the cuticle and others accumulating on the exocuticle. As hyphal proliferation continues, additional mucilage matrices are secreted near the attachment structures, facilitating the dissolution and erosion of the insect's cuticle.

Once inside, the hyphae breach the basal membrane and enter the hemocoel, where they proliferate throughout the host body. On the cuticle surface, the fungus differentiates into conidiophores, producing chains of colorless conidia that serve as secondary infection propagules. Eventually, mycelial outgrowths emerge from the aphid cadaver, enabling further dispersal. Proteolytic enzymes, such as Pr1 protease and chitinase, have been detected in culture media containing *C. javanica* conidia, indicating their involvement in host penetration and infection (Wang *et al*. 2023).

Insecticidal metabolites in *C. javanica* fermentation broth

Beyond enzymatic virulence factors, *C. javanica* produces insecticidal secondary metabolites, including small-molecule toxins such as heteratisine. Heteratisine is an alkaloid that binds to the peripheral anionic site of acetylcholinesterase, thereby inhibiting the enzyme's activity in aphids. This mode of action disrupts neurotransmission and contributes to the insecticidal effect of *C. javanica* fermentation broth (Xing *et al*. 2024).

Pathogenicity of *C. javanica* against various insect pests

Several studies have demonstrated the efficacy of *C. javanica* against a range of insect pests. *C. javanica* has shown significant pathogenicity against the Asian citrus psyllid (*Diaphorina citri* Kuwayama, 1908), a major vector of citrus greening disease (Meyer *et al*. 2008; Ou *et al*. 2019b; Awan *et al*. 2021). Additionally, Nguyen *et al*. (2017) isolated *C. javanica* strains highly virulent against the diamondback moth (*P. xylostella*) and the common cutworm (*S. litura*).

Compatibility of *C. javanica* with natural enemies in pest control

When applying *C. javanica* for field pest management, its impact on natural enemies must be carefully evaluated. Ou *et al*. (2019a) analyzed the pathogenicity of *C. javanica* against *Eretmocerus hayati* Zolnerowich & Rose, 1998, a parasitoid of the whitefly *Bemisia tabaci* (Gennadius, 1889). Their findings revealed that higher concentrations of *C. javanica* increased pathogenicity against *E. hayati*, but its impact

on the parasitoid was significantly lower than on *B. tabaci*. According to the International Organization for Biological Control (IOBC) standards, *C. javanica* was harmless to *E. hayati* pupae and only slightly harmful to adults. Interestingly, infection of *B. tabaci* nymphs with *C. javanica* extended the lifespan and developmental period of *E. hayati* offspring.

Semi-field trials demonstrated that the combined application of *C. javanica* and *E. hayati* resulted in a superior control of *B. tabaci* compared to either agent alone. These findings highlight the potential of integrating *C. javanica* with parasitoids for enhanced and compatible biological control strategies against *B. tabaci*.

ENTOMOPATHOGENIC VIRUSES

Baculoviridae: A major group of entomopathogenic viruses

The Baculoviridae family is widely used for insect pest control and primarily infects insects of the orders Lepidoptera, Hymenoptera, and Diptera. These large, double-stranded circular DNA viruses range from 80 to 180 kbp in size and contain 90 to 180 genes. Baculoviruses are characterized by their rod-shaped nucleocapsids enclosed within a lipid envelope and occlusion bodies (OBs) measuring 0.15−5 μm (Okano *et al*. 2006; Harrison *et al*. 2018; Sajid *et al*. 2021).

Classification based on occlusion bodymorphology

Baculoviruses are classified into two major types based on their OB structures: nucleopolyhedroviruses (NPVs) and granuloviruses (GVs). NPVs produce large OBs in the nuclei of infected cells, encapsulating multiple rod-shaped virions within a crystalline polyhedral protein matrix. These OBs, also known as polyhedral inclusion bodies, are smooth-surfaced. In contrast, GVs form small, granular OBs composed of granulin protein, each typically containing a single virion. GVs are only found in Lepidoptera (Winstanley & O'Reilly 1999; Williams *et al*. 2022). The virions within OBs are referred to as occlusion-derived viruses (ODVs), which consist of one or more rod-shaped nucleocapsids with unique structural polarity, enclosed within a membrane envelope (Harrison *et al*. 2018).

Baculovirus infection cycle

The infection cycle of baculoviruses produces two forms of virions: ODVs and budded viruses (BVs). Upon ingestion by a susceptible insect, the high alkalinity and proteolytic enzymes in the midgut dissolve the OBs, releasing ODVs. These ODVs penetrate the peritrophic membrane and fuse with the midgut epithelium cell membranes through interactions involving a multiprotein complex of per os infectivity factors (PIFs). The nucleocapsids enter the midgut epithelial cells, disrupting tissue integrity and ultimately liquefying the host (Fig. 3). (Kawanishi *et al*. 1972; Horton & Burand 1993; Javed *et al*. 2017; Erlandson *et al*. 2019; Wang *et al*. 2019).

BVs are produced when nucleocapsids bud from the infected cell membrane, spreading through the hemolymph and tracheal system to infect other tissues (Passarelli 2011). BVs typically contain a single nucleocapsid enclosed within a cytoplasmic membrane-derived envelope, incorporating envelope fusion glycoproteins (EFPs). These glycoproteins form dense spike-like projections on one or both ends of the virion (Wang *et al*. 2016). In the later stages of infection, ODVs are assembled and enclosed within the nucleus to form OBs.

Host manipulation and virus dissemination

Baculoviruses manipulate host behavior to enhance viral dissemination. Infected larvae exhibit climbing behavior before death, positioning themselves at the upper parts of the host plant. The viral enzyme ecdysteroid UDP-glucosyltransferase (EGT) inhibits the activation of molting hormones, preventing the larvae from molting (O'Reilly & Miller 1989). The cuticle of infected larvae is weakened by viral proteases, such as cathepsin-like protease

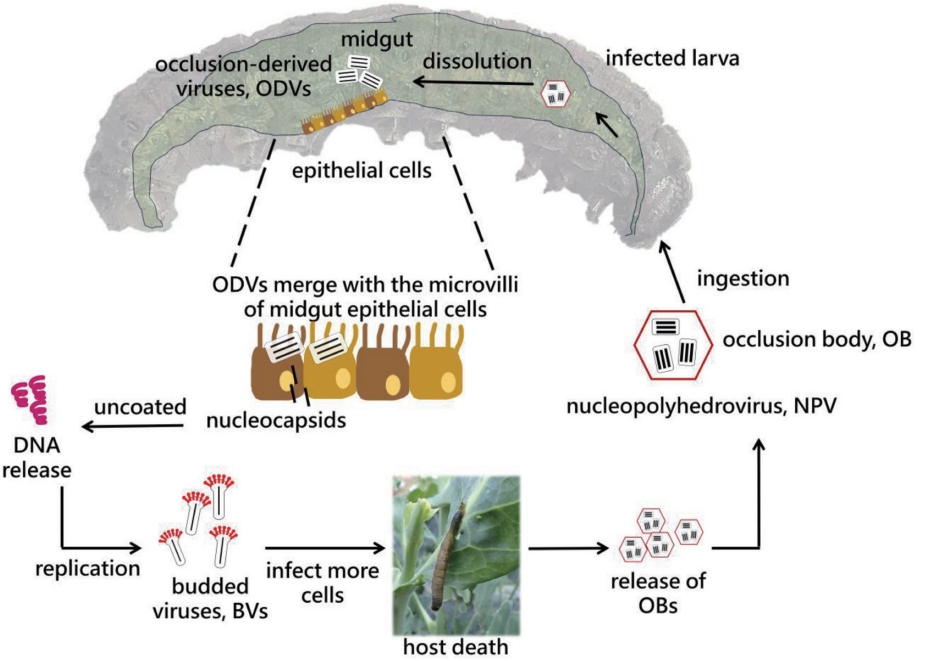

Fig. 3. Mode of action of nucleopolyhedroviruses in Lepidoptera.

and chitinase, leading to host liquefaction and the release of millions of OB progeny onto plant surfaces. This process ensures efficient viral transmission to new hosts (Hawtin *et al*. 1997).

Taxonomic classification and genetic characteristics of Baculoviridae

Baculoviridae is classified into four genera based on phylogenetic relationships, genomic characteristics, host range, and OB morphology: *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus*. These four genera share a total of 38 homeotic genes, which are highly conserved and involved in essential functions such as DNA replication, late gene transcription, and virion structure (Jehle *et al*. 2006; Garavaglia *et al*. 2012; Javed *et al*. 2017).

Members of the genus *Alphabaculovirus* infect lepidopteran larvae, with polyhedral OBs containing multiple virions. The occlusion-derived virus (ODV) undergoes nucleocapsid envelopment within the nucleus. Members of the genus *Betabaculovirus*, isolated only from lepidopteran larvae, form granular OBs, and ODV nucleocapsid envelopment occurs in the nuclear-cytoplasmic milieu after the nuclear membrane is lost. Both *Gammabaculovirus* and *Deltabaculovirus* exhibit polyhedral OBs containing multiple virions (Harrison *et al*. 2018).

Transmission and environmental persistence of baculoviruses

Baculoviruses spread among insects through both horizontal and vertical transmission. Horizontal transmission, the primary mode, occurs when insects ingest contaminated food or come into contact with OBs on egg surfaces. Vertical transmission involves the transfer of viruses from adult insects to their offspring via infected eggs (Doane 1969). Once a suitable host is infected, the virus undergoes a developmental cycle lasting approximately 1 wk, ultimately causing host liquefaction and releasing OBs into the environment. These OBs represent a stable form of the virus, exhibiting high resistance

to chemical and physical degradation, as well as UV inactivation. Additionally, studies have documented the ability of baculoviruses to persist asymptomatically in host populations (Myers & Cory 2016).

Host specificity and application in pest control

Compared to other entomopathogens such as EPFs and *B. thuringiensis*, baculoviruses exhibit exceptionally high host specificity. This trait significantly reduces non-target effects and minimizes ecological disruption when applied in the field (Armenta *et al*. 2003; Williams *et al*. 2022). However, since infected insects continue feeding for several days to weeks before death, they may still cause agricultural damage. To mitigate this issue, high concentrations of OBs or scheduled OB applications can target pests at their earliest developmental stages, reducing damage (Harper 1973). Additionally, genetic modification of baculoviruses to express insecticidal protein genes has been explored to accelerate pest mortality, inhibit feeding, or enhance overall virulence (Kroemer *et al*. 2015).

Behavioral manipulation by baculoviruses

Baculoviruses are known to alter host behavior post-infection. For instance, *L. dispar* larvae infected with baculoviruses exhibit altered nocturnal behavior, climbing to the tops of plants before death. This behavior, mediated by the EGT gene, ensures that liquefied cadavers release viral particles from elevated positions, enhancing transmission efficiency (Hoover *et al*. 2011). Additionally, viral protein tyrosine phosphatase (PTP) expression has been linked to increased host locomotor activity, promoting wandering behavior and further facilitating viral dispersal (Katsuma *et al*. 2012; van Houte *et al*. 2012). Phylogenetic analyses of PTP and EGT genes suggest that these genes may have originated from lepidopteran hosts, indicating that baculoviruses may have acquired advantageous genes during co-evolution with their hosts (Clem & Passarelli 2013).

Development of baculovirus-based biopesticides in Taiwan

In Taiwan, NPVs were isolated from *S. litura* and *S. exigua* larvae (Tuan *et al*. 1994, 1995). These viruses were highly pathogenic, inducing nuclear hypertrophy and tissue disintegration, with larval susceptibility decreasing with age. However, large-scale application was constrained by high production costs, as the virus had to be propagated in noctuid larvae prior to extraction. Traditional production methods involved grinding NPV-infected larvae, followed by viral extraction (Tuan *et al*. 1998). Alternatively, infected larvae could be frozen and processed when needed, although cold storage was found to reduce viral activity.

Recent advancements have overcome formulation challenges, resulting in a water-dispersible granule formulation that remains stable at room temperature, thereby greatly enhancing its usability (https://www.acri.gov.tw/Uploads/Item/14bc92c3-c799-4a8b-94c2-3c74ecf7b99b.pdf). Additionally, automated mass production has been integrated with AI technology. This system combines image recognition with robotic handling to automate insect rearing, viral infection, disease monitoring, viral extraction, and storage, drastically reducing labor costs and enhancing production efficiency (Deng *et al*. 2024).

Together, these developments demonstrate Taiwan's steady progress toward industrial-scale production and practical application of baculovirus-based biopesticides for sustainable pest management.

CONCLUSIONS

Pathogenic microorganisms hold significant potential for agricultural pest management, offering environmentally friendly solutions that enhance biodiversity and reduce reliance on chemical pesticides. Bacterial pathogens such as *B. thuringiensis*, as well as EPFs like *B. bassiana*, *M. anisopliae*, and *C. javanica* have demonstrated remarkable efficacy in pest control. Among these,

Bt has been widely adopted in agriculture due to its insect-specific toxic proteins and minimal impact on non-target organisms, playing a crucial role in managing pests affecting crops such as cotton and maize.

However, the use of microbial control agents still faces notable challenges, including the evolution of insect resistance, environmental variability affecting efficacy, and limitations in formulation stability and shelf life. Addressing these issues requires continuous innovation in strain improvement, formulation technology, and the strategic integration of microbial agents with complementary pest management tactics. Future research should focus on enhancing the virulence, stability, and field adaptability of these agents, while deepening our understanding of their ecological interactions within IPM systems. Such efforts will be key to realizing the full potential of microbial biopesticides as sustainable tools for modern agriculture.

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農業重要害蟲防治用病原微生物研究與發展

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摘要

張淑貞、李中潔、江明耀。2026。農業重要害蟲防治用病原微生物研究與發展。台灣農業研究 75(1):27–49。

病原性微生物在農業害蟲防治中具關鍵地位，能透過寄生、感染或產生毒素有效抑制害蟲族群，為化學農藥提供環境友善且永續的替代方案，並推動病蟲害綜合管理 (integrated pest management; IPM)。昆蟲病原微生物的研究與應用歷史悠久，常見的昆蟲病原細菌如蘇力菌 (*Bacillus thuringiensis*)、病原真菌如白殭菌 (*Beauveria bassiana*)、黑殭菌 (*Metarhizium anisopliae*)、爪哇擬青黴菌 (*Cordyceps javanica*)，及昆蟲病原病毒如核多角體病毒 (Nucleopolyhedrovirus; NPV)。這些微生物因專一性高、對環境安全且可降低農藥依賴，已成為 IPM 中的重要組成。近年基因體學與生物技術的進展，促進了高效菌株的篩選與毒力機制解析。全基因體定序與轉錄體分析使有助於鑑定致病相關基因，並揭示毒素生成、寄主感染與適應機制。此外，特定酵素如幾丁質酶、蛋白酶及次級代謝物的研究，亦提升病原微生物的感染力與殺蟲效能，擴大其應用潛力。然而，微生物防治仍面臨環境適應性、寄主抗性及製劑穩定性等挑戰。整體而言，病原微生物防治技術已成為現代農業的重要方向，隨著生物技術與應用策略的持續進步，將有望成為永續害蟲管理與糧食安全的核心支柱。

關鍵詞：昆蟲病原微生物、微生物農藥、細菌、真菌、病毒。

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